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Potential for Selective Activity of the ALS Inhibitors Penoxsulam, Bispyribac-sodium, and Imazamox on Algae Responsible for Harmful Blooms

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INTRODUCTION

In 2007, there were 45 acetolactate synthase (ALS) inhibiting herbicides registered for use by the U.S. Environmental Protection Agency (USEPA; WSSA 2007). The registration of such a large number of herbicides with the same mode of action is based largely on the differential selectivity exhibited by these products. Slight changes in the molecular structure of ALS-inhibiting herbicides can greatly affect their potency and weed control spectrum (Ren et al. 2000). The selectivity of the ALS herbicides is due to the ability of different plant species to metabolize the herbicide, or in some cases tolerant plants can have an altered target enzyme site. The ALS herbicides are widely used in cropping, forestry, right of way, and aquatic sites. From an aquatic registration perspective, ALS inhibitors exhibit a high margin of safety for fish and wildlife, and mesocosm screening suggests they can be selective against numerous aquatic macrophyte species (Nelson et al.

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1993, Getsinger et al. 1994, Chiconela et al. 2004, Koschnick et al. 2007, Glomski and Netherland 2008). Algae and cyanobacteria have many of the same enzyme systems as higher plants, and therefore the question was raised as to whether some of the enzyme-specific inhibiting herbicides may be active against certain algal species responsible for harmful blooms.

Harmful algal blooms (HAB) are expanding from tropical to temperate waters, and the factors that contribute to their bloom formation, toxin production, and toxin release have proven to be multifaceted and poorly understood (Dokulil and Teubner 2000). Several species recognized as being problematic bloom formers include *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa*, *Oscillatoria perornata*, *Prymnesium parvum*, and *Pseudanabaena limnetica* (Poovey and Netherland 2006). These algae are known to produce neurotoxins (i.e., endotoxins, anatoxins) and hepatoxins (i.e., microcystins and cylindrospermopsins). Harmful algal blooms have been associated with degraded water quality and can jeopardize aquatic ecosystem health by causing fish and wildlife mortality or impeding growth of aquatic flora or fauna (Casanova et al. 1999, Lindholm et al. 1999, Oberemm et al. 1999, LeBlanc et al. 2005, Wilde et al. 2005).

Identification and evaluation of potential new organic algaecides has received minimal research attention, and as a result algal control efforts continue to rely heavily on the use of broad-spectrum copper-based compounds (Murray-Gulde et al. 2002). While copper use has many benefits (no restrictions on use in potable water, cost-effective control), broad-spectrum control of algae can lead to depressed dissolved oxygen levels or toxin release (Jones and Orr 1994). There is also evidence of potential selection for more copper-tolerant algae (Lembi 2000), suggesting development of an alternative mode of action would be warranted.

In past decades photosynthetic-inhibiting herbicides, such as diuron and simazine, were registered for use as broad-spectrum algaecides for smaller water bodies. The photosynthetic inhibitor diquat (currently registered for use as an aquatic herbicide) has also been tested against various cyanobacteria species (Phlips et al. 1992). None of these herbicides are currently registered to control HAB in public waters. Due to a lack of viable alternatives, diuron has been granted a Section 18 emergency use label issued by the USEPA to control algae (mainly *Oscillatoria perornata)* that cause taste and odor problems in catfish ponds (Schrader et al. 1998, Tucker 2000, Schrader and Harries 2001). Because diuron is a broad-spectrum product with potential environmental and human health concerns, there are ongoing research efforts by United States Department of Agriculture (USDA) scientists to find and evaluate natural and reduced-risk compounds for effective and selective algae control in catfish farming (Schrader and Harries 2001, Schrader et al. 2003, 2004, Schrader 2005).

The recent development and aquatic registration of highly selective ALS herbicides presents an opportunity to investigate the algicidal properties of these compounds. Like copper, the current aquatic labels for these ALS herbicides do not include use restrictions for drinking, swimming, and fishing. Based on selective properties in terrestrial systems, we hypothesized these enzyme-specific inhibitors may show a high level of specificity for certain species of problem cyanobacteria and algae. In this study, we sought to determine the activity of the USEPA-registered ALS inhibiting herbicides penoxsulam [2-(2,2-difluoroethoxy)-N-(5,8-dimethoxy[1,2,4] triazolo[1,5-c]pyrimidin-2-yl)-6 (trifluoromethyl) benzenesulfonamide] and imazamox [2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid], and the Experimental Use Permit herbicide bispyribac-sodium [2,6-bis(4,6-dimethoxypyrimidin-2-yloxy)benzoic acid] against several cyanobacteria associated with harmful blooms as well as species of green algae that are typically considered beneficial.

MATERIALS AND METHODS

All algae used in efficacy screens were maintained in unialgal culture in an inorganic liquid medium appropriate for culture of that species. Studies were conducted in 500-ml Erlenmeyer flasks filled with 200 ml of an appropriate culture medium, the species of interest, and a known concentration of herbicide. Flasks were placed in a controlled-environment growth chamber (Percival E36 L) at 65 to 120 umol/m^2/sec (depending on species), 25 C, and a 16:8 h light:dark photoperiod.

The activity of ALS inhibitors penoxsulam, bispyribac sodium, and imazamox was screened under the culture growth conditions described above at use rates of 100, 200, and 500 µg active ingredient (ai) L^{-1} for activity against the planktonic blue-green algae *Anabaena* sp, *Cylindrospermopsis raciborskii, Microcystis aeruginosa*, and *Pseudanabaena limnetica*. In addition to algae associated with harmful blooms, we also screened activity against the beneficial planktonic green algae *Ankistrodesmus falcatus*, *Scenedesmus quadricauda*, and *Selenastrum* sp.

After a 2-week exposure period to the ALS inhibitors, all flasks were filtered. The planktonic algae were measured for chlorophyll-*a* content prior to treatment (referred to as initials) and at the conclusion of a 2-week exposure period. All treatments were replicated four times. Chlorophyll data are presented as mean values with 95% confidence intervals.

RESULTS AND DISCUSSION

All algal species grew readily during the course of the treatments as evidenced by the increase in chlorophyll content between initial and untreated reference flasks (Figure 1). Active growth in these screens is important because the activity of ALS-inhibiting compounds is enhanced when enzyme turnover is increased during rapid growth (WSSA 2007). Although bispyribac did show evidence of activity against the green alga S*cenedesmus*, screening of bispyribac and imazamox suggests these products have limited activity against the other algal species tested (Figure 1).

Penoxsulam was highly active against *Cylindrospermopsis* and *Anabaena,* as well as the green algae *Scenedesmus*, with the lowest dose of 100 µg ai L^{-1} providing >90% chlorophyll reduction (Figure 1). A 100 µg ai L^{-1} penoxsulam treatment reduced *Pseudanabaena* chlorophyll-*a* content by 58%, while concentrations of 200 and 500 µg ai L^{-1} reduced chlorophyll-*a* levels by 85 and 90%, respectively. Penoxsulam did not reduce chlorophyll-*a* for the noxious cyanobacterium *Microcystis* or the green algae *Ankistrodesmus* and *Selenastrum.*

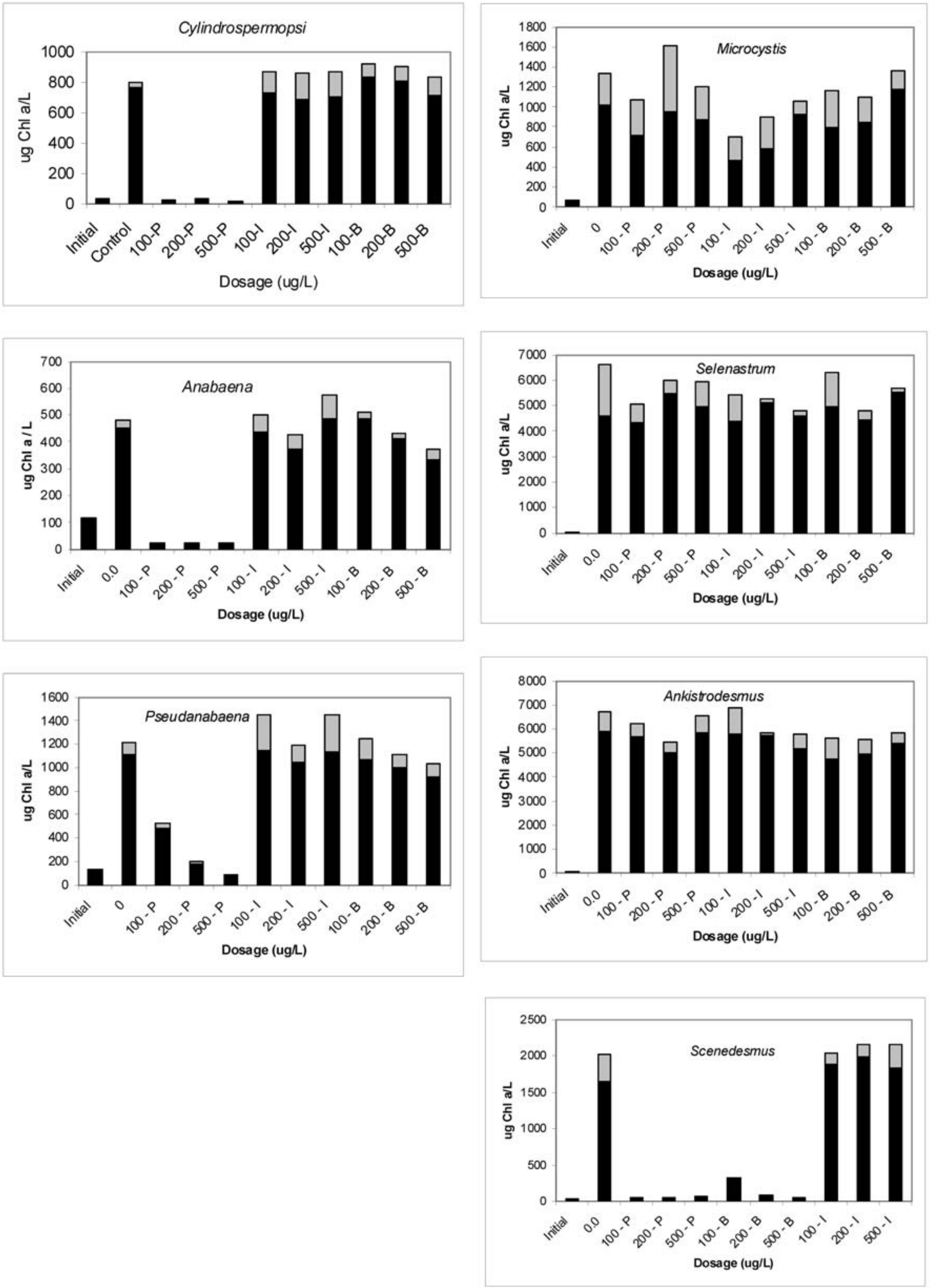

Figure 1. Chlorophyll *a* response of unialgal cultures of seven species to the ALS-inhibiting herbicides penoxsulam (P), imazamox (I) and bispyribac (B). Treatment rates were 100, 200, and 500 µg active ingredient (ai) L^{-1}. Each bar represents the mean of 4 replicate treatments with 95% confidence intervals

This differential selectivity for these three ALS herbicides on various algal and cyanobacterial species is consistent with selectivity patterns demonstrated for both emergent and submersed aquatic macrophytes (Koschnick et al. 2007, Glomski and Netherland 2008). Although current recommended use rates for the ALS compounds are typically lower than the 100 µg ai L^{-1} concentration we evaluated (current use rates range from 10 to 75 µg ai L^{-1}), the intent of these laboratory trials was to determine which compounds displayed activity against algae associated with harmful blooms. The short-term nature of these assays (14 days) was meant to predict potential activity and not meant to mimic results of longer-term field exposures.

While penoxsulam and bispyribac each impacted a green algae, there was no indication that any of the ALS compounds would be active against a broad range of green algae. Differential response of green algae to ALS-inhibiting herbicides has been documented in previous laboratory and field trials (Thompson et al. 1993, Nyström and Blanck 1998, Wei et al. 1998). Further testing with different target and beneficial algal species is needed to determine direct impacts of these herbicides on different algal classes (e.g., diatoms and filamentous algae).

The inability to identify environmentally compatible, cost-effective, and selective alternatives to copper over the past several decades has contributed to skepticism regarding the development of new algaecides. Management of invasive aquatic macrophytes has dominated research efforts with newly registered herbicides, yet these compounds may provide potential tools for addressing problems with HAB. Developing new, cost-effective, and environmentally compatible algaecides will require an innovative approach to adequately protect water resources. The lack of broad-spectrum algal control provided by these enzyme-specific herbicides can be viewed as a limitation for future development; however, these data suggest the potential for selective control of algae responsible for nuisance or harmful blooms. Many of the newer classes of herbicides that target specific plant enzyme systems have toxicology packages compatible for large-scale use without associated water use restrictions. Results of this initial testing suggest that evaluations of other enzyme-specific herbicides are warranted to determine selectivity patterns and potentially refine use rates and exposure times for target algae.

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